

Experimental Researches on Vegetable Assimilation and Respiration. XIV.—*Assimilation by Submerged Plants in Dilute Solutions of Bicarbonates and of Acids: an Improved Bubble-Counting Technique.*

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The procedure of counting the bubbles given off from the cut stem of a submerged water plant, to obtain a measure of the magnitude of the plant's assimilation, was introduced by Dutrochet in 1837 and matured by Sachs in 1864. It has been of great use in demonstrations and class work, and has also been seriously employed in a number of researches. From time to time it has been subjected to a good deal of criticism, as giving a faulty measure of the true rate of photosynthesis. It is, however, a striking fact that nothing has been done to improve the technique of the procedure since the method was originally introduced.

The first part of the present paper describes a simple device which removes at once two of the very serious defects of the method, and renders it much more suitable for research work. The second part applies this method to an elucidation of the extraordinary effect of dilute mineral acids upon bubble rate brought forward by Treboux. In the third part the relation of bubbling in bicarbonates to bubbling in carbonic acid is investigated, and it is shown that Angelstein's statement that water plants can actively split bicarbonates in solution is erroneous.

Part I.—THE BUBBLE-COUNTING METHOD OF MEASURING CARBON-ASSIMILATION IN SUBMERGED PLANTS.

During the course of the investigations described in the later parts of this paper, which were carried out in the Botany School, Cambridge, in 1911, at the suggestion of Dr. F. F. Blackman, the bubble-counting method was carefully examined, and improved in several respects.

Various defects of this method had been pointed out before the present work, and, since that date, Kniep, in 1915, has destructively criticised it. None of its critics seems, however, to have attempted to improve it. Yet the method is so extraordinarily sensitive to influences of all sorts that it cannot be lightly abandoned as a research instrument. As will be shown immediately, some of its most serious defects are quite easily remedied in simple ways. These may be considered under two headings: (A) Methods of

avoiding errors in bubble-counting due to variations in size of the gas bubbles, and (B) Method of diminishing errors due to gaseous diffusion in the water round the plant.

(A) *Errors due to Variation in Size of Bubbles can be Avoided by the Use of Glass "Bubblers" and Bubbling Cup.*

Aa. *Variations in Size of Bubbles due to Alterations of Cut Stem Aperture.*—It is obvious that if, over a period of time, the size of the bubbles given off from the cut stem of a submerged water plant varies, either irregularly or by steady drift, then merely counting the number of bubbles gives no adequate measure of the volume of gas set free by the photosynthetic process. The papers of all workers who have used the bubble-counting method contain lamentations about the difficulty that this spontaneous variation produces in attempting long comparative experiments, yet no proposal has been brought forward for really overcoming it. Palliatives, such as cutting the surface overnight, pinching, scratching, covering with varnish and pricking, and so on, have been employed, but it is generally admitted that many experiments suffer from such obvious variations of bubble-size that they must be abandoned.

In the course of the experiments now described, it was found practicable to manufacture small glass nozzles to fit over the cut end of the shoot, and provide a fine glass capillary opening of absolutely constant size. These "bubblers," as they will be called, were made by first drawing out glass tubing to an external diameter of 1.5 to 2 mm., with an internal bore just sufficient to fit closely over the shoots to be experimented with. A short region of this tube was then sharply drawn out at right angles to a very fine capillary and broken off, to leave a minute aperture. Fitting such nozzles on to the cut stem ensures a long steady succession of bubbles all of the same size.

Ab. *Variations in Size of Bubbles with Alteration of Internal Gas-Pressure.*—Such changes are well known when the raw end of the plant is used. The openings of the plant capillary spaces are so fine that greatly increased rate of bubbling delivers smaller bubbles than does a slow flow of gas, and thus quite invalidates the counted numbers of bubbles. This source of error is also eliminated by the glass bubblers, because the apertures are larger than in such plants as *Elodea*, and the bubbles delivered are larger and come off much more slowly, so that a rate of flow which would affect their individual size is never reached in normal photosynthesis.

A nozzle that gives a bubble every 5 to 10 seconds is the most satisfactory size. It is found that, in constant conditions, the variation of timing five

bubbles may not exceed 2 per cent. for several hours. Bubbler delivering even as slowly as one bubble in 8·5 minutes gave constant results to within 2 seconds.

One very great advantage of these bubblers is that the same one may be used for different plants in succession, and so strict comparisons made independently of the size of the plant's own capillary openings.

Unless the bubblers are quite clean, the bubbles may tend to cling to the tip. This can be cured by boiling them in a test-tube with distilled water.

With the comparatively large slow bubbles of the glass bubbler, it is easy to see that the range of natural rates does not affect the size of the bubbles. One never gets an almost continuous stream of very tiny bubbles, but, after each escapes, the water springs back a little way into the capillary nozzle and remains stationary for a bit while the internal pressure gets up; then it is quickly pushed out to give a nearly full-sized bubble, after which the bubble slowly increases to full size against the surface tension till the critical point is reached and it breaks away. This pulsating rhythm is repeated with great steadiness.

Ac. Variations of Size of Bubbles due to Varying Surface-Tension of the Solution.—A line of research, for which the bubble method has been used by Treboux, Jacobi, Pantanelli and others, is the study of the effect, on the rate of photosynthesis, of substances added to the water so as to penetrate into the plant. Any alteration of surface-tension of the water by these solutes must directly affect the size of the bubbles given off, so that bubble-counting ceases to give an accurate measure of the change produced in the assimilating cells. This complication can be completely avoided by the use of another simple device. The bubbles are in our experiments delivered not into the body of the experimental liquid, but into a small cup which contains distilled water, so that the tension is unaffected when solutions are changed. This cup is shown, together with the bubbler, in fig. 1. It consists of a glass tube 18 mm. wide, closed below by a cork, which has a hole through which the inverted plant stem is pushed, and secured above and below with a tenacious wax mixture of low melting point. The shoot is first attached to the cork and bubbler, and then the outer tube is slipped down on to the cork to form the bubbling cup.

Ad. Variations of Size of Bubbles due to Osmotic Action of Liquids on the Stem Aperture.—Pantanelli, Treboux and others have set out to investigate the effect of quite strong solutions of various salts upon photosynthesis in water plants. It is clear that, without special precautions, such solutions must shrink the cells bounding the stem aperture, and so enlarge the aperture, and probably the size of the bubbles.

That this effect really exists was demonstrated by some special experiments in which air was forced slowly by pressure through a short length of Elodea stem (one or two internodes), so that it escaped in bubbles at the free end. The other end was connected by rubber and glass tubing to a head of water forcing air through the plant. A scale was placed behind one of the intervening tubes, and the travel of the air-water meniscus at the head of the

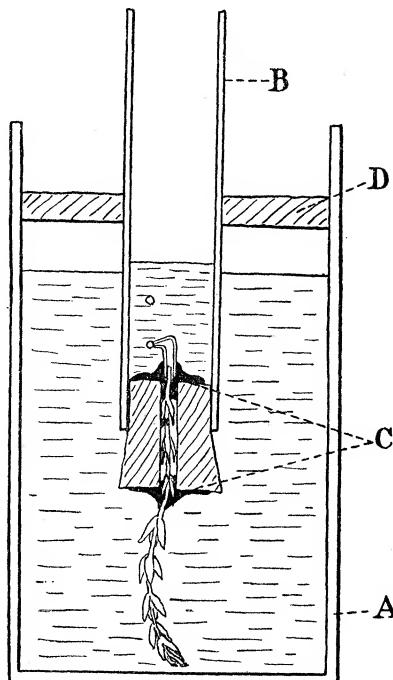


FIG. 1.—Fig. 1 shows the arrangement of the parts during experiments. A, flat-sided glass museum jar, containing the experimental solution; B, "bubbling cup," containing distilled water, closed by cork below; C, wax mixture, which supports the shoot in the cork and completely separates the liquids in A and B; D, cork lid, which hinders the loss of CO_2 from the top of the solution and supports the bubbling cup and also a thermometer. The glass "bubbler" is shown attached to the cut end of the shoot with a bubble escaping from it. [In the figure, to save space, the shoot and the jar, A, are drawn much too small and short relatively to the essential central parts.]

column of water was read for a given number of bubbles. When the index column of water had reached the end of the scale, the connection was interrupted and the index sucked back, so that further readings could be taken after re-connecting. The whole was so arranged that the final glass tube carrying the Elodea shoot, came up through a cork in the bottom of a vessel into which various liquids could be introduced.

It was demonstrated that the bubbles have a larger volume when the vessel contains osmotically active salt solutions than when water is used. In a whole shoot of *Elodea* carrying on assimilation in a salt solution the effect would be recorded as a smaller number of bubbles in unit time, which is what Treboux found in his Experiments 1 to 6. On replacing the salt solution by distilled water, the cells, now containing salt, absorb water and swell round the aperture, reducing its size and also that of the bubbles, and sometimes (after strong solutions) completely stopping bubbling by closing up the aperture.

With this apparatus was also demonstrated the effect of changed surface-tension. Thus, on adding methylated spirit to the vessel the bubble size is decreased, which in natural experimentation would express itself as an increased rate of bubbling, such as Darwin and Pertz observed. This smaller size of bubbles on adding methylated spirit was produced (to about 10 per cent.) also when a glass bubbler was fixed to the free end of the *Elodea* stem, and air forced through the whole system.

The mechanism of bubble-initiation, swelling, and liberation with the glass bubbler, as described above, was followed carefully with this apparatus at various rates, and as produced by different heads of water. In some cases a slight head of water produced no bubbles, but increasing pressure caused a sudden rapid rush of bubbles, which quickly slackened and then died completely away. Further increase caused a repetition of this without any regular bubbling occurring. This irregularity was evidently caused by the formation of water films across all the intercellular spaces, bubbling occurring only when the pressure became sufficient to burst them, after which they re-formed. These same "ruses of bubbles" occurred also with material used in these experiments, seeming to be most frequent during very dull weather. Probably during very dull spring days the respiration may exceed the assimilation, the volume of the internal air system being reduced until water films are formed. Sometimes a plant which at first refused to bubble would begin in the middle of an experiment (two shoots side by side were used to ensure one of them finishing the experiment) and either bubble regularly or in "ruses."

Adopting the bubblers and bubbling cup described in this section, we have every reason to believe that all the sources of error in bubble-counting which are due to variation in size of bubbles have been successfully removed, so that simple counting becomes a direct measure of volume of gas leaving the shoot, even when different plants are used in a series of experiments. There is now another, more subtle source of complications to be considered.

(B) *Method of Diminishing Errors due to Gaseous Diffusion in the Water round the Plant.*

As the gaseous exchange of the assimilating plant takes place in the centre of a mass of water, its supply of CO_2 , when the water is not in movement, depends upon diffusion from all around the plant. This factor is particularly important in laboratory experiments with vessels of very still water, and but slight temperature-convection currents. Certain effects due to this cause have not always been rightly understood. Similarly with the oxygen produced: this may leave the plant by the path of diffusion into the water as well as by escape through the intercellular spaces. Both these matters have been sources of illusion in early experiments.

Ba. *Aberrations due to Deficient Oxygen in Solution: the "Initial Oxygen Diffusion Effect."*—The less oxygen there is in solution in the water the greater must be the proportion of photosynthetic oxygen which diffuses away into the water and fails to record itself as bubbles. Most observers have used for their work water in which the oxygen in solution is no greater than that due to equilibrium with the atmosphere, so that the water against the plant only contains initially 21 per cent. of the volume of oxygen it can hold in solution in equilibrium with pure oxygen. In such a medium, when the plant is first illuminated, the bubbling rate is very slow, but steadily increases, more and more of the oxygen escaping as bubbles. The increase is at first rapid and then slows up smoothly towards an approximately constant rate, reached when only a little is passing away by diffusion. Fig. 2 gives an excellent illustration of the effect. These data are taken from Angelstein (p. 97). The nearly level rate that is obtained in due time is not absolutely stable, but depends on a steady diffusion gradient being maintained. Any small mechanical shake is liable to disturb it. Stirring brings the bubble rate down instantaneously and then it slowly climbs up again. This phenomenon has necessarily been encountered by all workers. Some have discarded these initial periods altogether, some recorded them as disturbing factors without explanation, and others have interpreted them correctly, but no one seems to have attempted to eliminate them for their research work, systematically, by charging the solutions to be used with a larger amount of oxygen. In working on the effect of drugs and other solutes on bubbling plants time is precious and the initial period cannot well be spared.

In the work on effects of acids and bicarbonates which follows, water was used which was charged with oxygen by prolonged violent shaking in bottles with an atmosphere of pure oxygen from a cylinder. The charging process is very slow in its later stages. Such water was used for diluting the standard

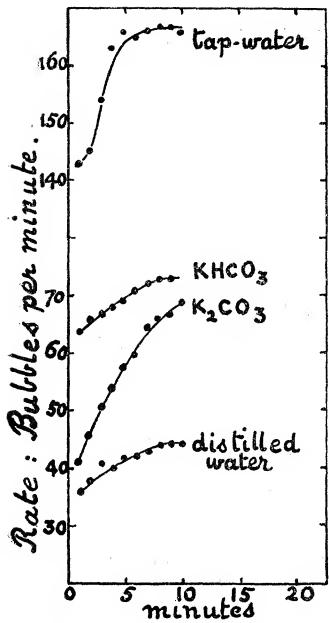


FIG. 2.

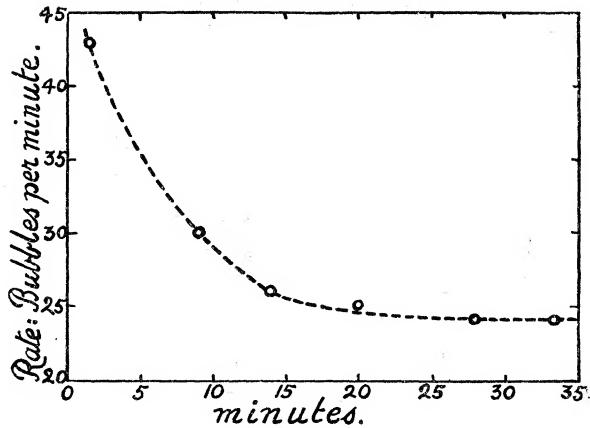


FIG. 3.

FIG. 2.—Initial oxygen diffusion effect plotted from Angelstein's experiments. Four solutions, respectively distilled water, dilute KHCO₃, dilute K₂CO₃, and tap water, were allowed to stand in shallow dishes in the air for fourteen days that their CO₂-content might come into equilibrium with the air, and then the rates of bubbling of Elodea were measured in them. It is obvious that during the initial period here figured, which is all Angelstein records, the bubble rates, rising rapidly as they do, do not measure the CO₂-content, but merely the building up of the oxygen diffusion gradients in the solutions.

FIG. 3.—"Initial CO₂ diffusion effect" with Elodea set up in a low concentration of CO₂,

strong CO₂ solutions before use; for the sodium bicarbonate solutions the solid salt was dissolved directly in it. The test of its efficiency was that when bubbling was started no such initial oxygen diffusion effects as are here described were to be observed.

Bb. *Carbon Dioxide in Solution: the "Initial CO₂ Diffusion Effect."*—We have now to deal with a second aberration depending on diffusion that characterises the initial stages of bubbling in a solution. When a plant is first put to bubble in a solution containing CO₂ the layers against the plant are charged with this solute to the same extent as the general body of the fluid. As photosynthesis proceeds they are robbed of CO₂ and a diffusion gradient is gradually built up, supplying fresh CO₂ from the more remote layers. The effective concentration, then, is never so great again as at the first moment. If the CO₂ concentration is not very great compared with the

intensity of the other factors, so that CO_2 is a limiting factor in Blackman's sense, then the photosynthetic rate will necessarily be high at first and steadily decline as the diffusion gradient slackens, until an approximately steady gradient is established, bringing along CO_2 at the rate which is that appropriate to the general concentration. Here we have an initial aberration which is exactly the inverse of the oxygen aberration. This is illustrated in fig. 3, drawn from the present work. This phenomenon repeats itself after every movement or stirring, which brings up more highly charged layers of CO_2 solution, and also after every period of darkening during which CO_2 has again accumulated round the plant. This type of initial effect has also been often observed, but hardly ever precisely interpreted.

It is interesting to note that it only occurs when the conditions of experimentation are such that CO_2 is limiting. When weak light is limiting and the CO_2 concentration is in excess, these variations of CO_2 -supply cannot, by definition, manifest themselves as changes of bubble rate, and it is found throughout the present work that the falling initial curve occurs only in the one class of cases and not in the other.

This initial aberration cannot be removed by any preliminary treatment. If investigation is to be made on the relation of assimilation to CO_2 concentration or to KHCO_3 solutions, this effect is bound up with the problem stated. The only theoretical way would be to use solutions which were always in violent movement, so that there were practically no layers less charged with CO_2 than their neighbours, but in such cases it would be very difficult to keep the lighting of the shoot absolutely constant.

Bc. Combination of Initial Oxygen Diffusion Effect with Initial CO_2 Diffusion Effect.—We now see the experimenter is faced with two initial effects of entirely opposite nature, one giving a rising series of readings and the other a falling series. The combination of them suggests interesting complications as each is independent of the other. It would be possible for the two to neutralise one another if the solution started with exactly the right deficit of oxygen and excess of CO_2 . It seems highly probable that in the cases here and there in the literature where the bubble rate starts at once at the rate that it continues to maintain we have such a relation. What appears to be an ideal experiment will then be only a chance cancelling out of two unmeasured sources of error.

The experiments in this paper show big CO_2 effects as compared with other workers because special trouble was taken to eliminate the oxygen effect. No one is directly interested in the relation of bubble rate to the oxygen-content of the water, and everyone in its relation to the CO_2 -content, so the best procedure seems to be to eliminate one source of error and so get a clear measure of the magnitude of the other source.

In the following experiments the initial CO_2 effect occurred so regularly and was so obvious to the eye that readings were often omitted during it. In the graphs of many of the experiments figured in this paper it will be seen that the curve of the initial phase has been drawn in without any actual data.

At the end of the initial phase (some 10 to 20 minutes) the bubble rates become practically constant for a long time, and it is these long constant values which are taken as the measure of the CO_2 assimilation possible when the CO_2 in solution or the CO_2 of bicarbonates is limiting. When the investigator changes the conditions every 10 minutes, as Pantanelli sometimes did, it is clear that the results recorded may be involved in considerable complications. Earlier observers have tried to rush through their experiments as quickly as possible to avoid spontaneous uncontrollable variations in bubble size, but the use of glass bubblers removes all this risk, and experiments can be carried out slowly and constancy assured in each condition before it is changed.

The uniformity of bubble rate is so regular a happening when a bubbler is used that the observer gets into the habit of taking many fewer readings of bubble rate than would be permissible with a varying cut stem. It will be noticed that in many of the graphs the actual points recorded seem rather scanty, but they are really quite adequate to establish the conclusions drawn when working with this improved technique.

(C) *Apparatus, Experimental Procedure, and Minor Sources of Error.*

The plants used in subsequent experiments were either *Elodea* or *Callitricha*. They were fitted with a glass bubbler and set up, depending from the cork of the bubbling cup in the way shown in fig. 1. The bubbling cup always contained distilled water, and it was fitted into a hole in a thick cork plate, which again fitted into the rectangular glass museum cell used as a plant chamber. For single shoots a chamber of 200 c.c. capacity was used. A hole in the plate near one corner, closed by a cork during experiment, allowed fresh solutions to be poured in through a funnel, while a small piece cut from the opposite corner allowed the liquid to be quickly poured away. In the experiments with acid the required amount of N/10 HCl was added through this hole from a graduated pipette, and stirred by twirling a glass rod which passed to the bottom of the cell and had its lower inch bent at right angles and flattened. The temperature could be read continuously from a thermometer passing through another hole in the lid.

The CO_2 solutions used were diluted from a stock standard containing about 12 volumes per cent. CO_2 , prepared from a Kipp's apparatus and cleansed from acid in the usual way; the actual strength was determined by titration with excess of baryta and hydrochloric acid. For the avoidance of

the initial oxygen diffusion effect solutions were made up as required by diluting this stock with water richly charged with oxygen, as already described. In the preparation of bicarbonate solutions the exact amount of sodium bicarbonate required was weighed out each time and dissolved in the oxygenated water just the moment before it was used.

Many shoots of water plants are curved near the tip. It is important to set up the shoot with the plane of the curvature at right angles to the light rays, so that the distance from the light remains constant after any re-setting of the shoot or alteration of its curvature.

Artificial light is needed for prolonged uniform illumination. To obtain strong enough light some observers have placed cylindrical vessels of water in front of the water-chamber containing the plant. These condense the light, as lenses, into a vertical strip upon the shoot. Experiments with this type of apparatus showed that the rates of bubbling were very sensitive to the slightest displacement of the cylinder or of the plant. A variation of the rate of bubbling by as much as 100 per cent. might be produced by shifting the plant 1 to 2 cm. laterally while the rest of the apparatus was unmoved. Such condensing cylinders were therefore abandoned. The light used in these experiments was that of a cluster of three large incandescent mantles fed with gas under a pressure of 8 inches of water (the Keith light). This gives a radiating surface about 3 inches wide by 5 inches high, and is very suitable and steady. The three burners are grouped on an equi-lateral triangle plan and the distance of the plant from the centre of this triangle is recorded. In some cases, where it has any significance, the intensity of illumination is expressed by calculation on the "inverse square of the distance" basis. The intensity at a distance of 10 cm. is arbitrarily taken as equal to 10 units. This law does not, of course, apply with any accuracy to so large a source of light.

If the temperature of the water and plant rise much during experimentation, errors of various natures, biological and physical, will creep into the readings. It is important to have an effective running water screen in front of the plant chamber to cut out most of the rays absorbable by glass and water, but it is inevitable that the shoot should be somewhat warmed by the additional radiation that it absorbs. In these experiments the experimental solutions did not rise in temperature more than $\frac{1}{2}^{\circ}$ to 1° C. The temperature of all the experiments lay between 15° and 18° C.

The rate of bubbling was timed at intervals with a stop-watch. The number of readings shown on the figures may appear rather scanty, but bubbles came off so regularly and slowly that each point recorded is in reality a series of as many readings as bubbles observed.

Part II.—ASSIMILATION BY ELODEA IN DILUTE SOLUTIONS OF HYDRO-CHLORIC ACID.

Treboux (1903) tried the effects of many solutes upon the rate of bubbling of submerged plants, and he found that they all had a depressant effect, except one class of substances—the organic and inorganic acids—which increased the bubbling rate considerably. Treboux does not seem to have considered this at all an amazing result, but simply analogises these acids with carbonic acid, which, of course, also produces the same consequence. At the suggestion of Dr. Blackman, I took up this problem to test whether this general action of acids is due to any direct action on the cell, or merely to their setting free carbonic acid from the plant, and so leading to an increased assimilation. Hydrochloric acid was selected for experiment, and it soon became clear that its action is of the indirect nature here suggested. All this work was done in 1911. The only modern comment on Treboux's results that has been noted is by Willstätter (1918, pp. 53-4), who thinks it improbable that they are due to a direct action on the photosynthetic mechanism. He suggests that it is more probable that the acid acts on the CO_2 -adsorbing property of living tissues, an effect which he has studied, displacing CO_2 , which may increase the bubbling, partly by escaping as a constituent of them, and partly by augmenting assimilation.

Treboux's observations with acids were numerous, and his effect is perfectly well established, though he notes that some other observers have failed to detect it. A single example will illustrate his results (Treboux, Experiment 36). The bubble rate was observed in water containing 0.3 per cent. CO_2 , and then increasing quantities of acid were added, the increase of bubble rate being recorded for each stage: finally, the plant was returned to CO_2 solution without acid. The solutions and rates are as follows:—

Concentration HCl in addition to 0.3 per cent. CO_2	Nil	0.0001 N	0.0002 N	0.0003 N	0.0004 N	Nil
Bubble rate per minute	26	46	66	86	106	26

Were Elodea one of those water plants which become in time obviously encrusted with chalk, explanation of the action of acids would be simple, but to the eye there is no sign of such incrustation.

Experimental Results.

Experiments were first made in spring with Elodea that had been growing all the winter in an open wooden tub, the water in which was largely rain water.

Figs. 4 and 5 show that this material does not exhibit the effect described by Treboux. Neither acidification increases the bubble rate appreciably. In fig. 5 (A) the early rate is lower than the later: this appeared possibly due to gas-pressure variation. Such instances of variation of rate without change of conditions were extremely rare: at such times the light did not appear to the eye to be as perfectly steady as usual.

The next step was to obtain Elodea from a natural water, and in April experiments were made with material from a chalky stream (at "Nine Wells"). Here the result is quite different, as figs. 6 and 7 show.

In fig. 6 the addition of acid about doubles the rate of bubbling in B. On setting up the plant in the unacidified CO_2 solution, it returns in D to its original rate, to be again accelerated by acid. At C it is seen that stirring causes a new transient "initial CO_2 diffusion effect," with return to the previous rate. If any of the increased bubble rate in B had been due to making good a previous lack of oxygen in the solution, stirring would have brought on a sudden drop, followed by a rising series of bubble rates, instead of the sudden rise with a falling series, characteristic of the CO_2 diffusion effect.

In fig. 7 we have at first a repetition of the effect of fig. 6. The final part provides a demonstration that the effect of added HCl is really due to liberated CO_2 , on applying the theory of limiting factors. If enough CO_2 is present in solution, then the rate of bubbling will be controlled by the light only as a limiting factor, and in such case, by definition, a further addition of CO_2 should not increase the rate of assimilation. If, now, all that HCl does is to generate more CO_2 locally at the surface of the plant, then in such a case, with CO_2 already in excess, addition of HCl should not increase the bubble rate. This is what is found at E. It should be noted that the "initial CO_2 diffusion effect" is also absent in D and E, and is very small, partly controlled by the light limit, at the beginning of B. We get thus another line of proof of the nature of the acidification effect.

We may safely conclude that Elodea, in chalky streams, does become impregnated at its surface with CaCO_3 , though not enough to be visibly affected, and that, after growing for some time in soft water, the shoots lose this calcification. The Elodea grown in a tub owes its inability to give an "acid effect" to this cause, and it was further proved that, when the material from the chalky stream was placed, in April, to grow in the tub, it ceased after two or three weeks to give this effect any longer (see Experiment table on p. 318).

Some further illustrations of the presence of the acid effect with fresh material when CO_2 is limiting and its absence when light is limiting are afforded by the next two experiments.

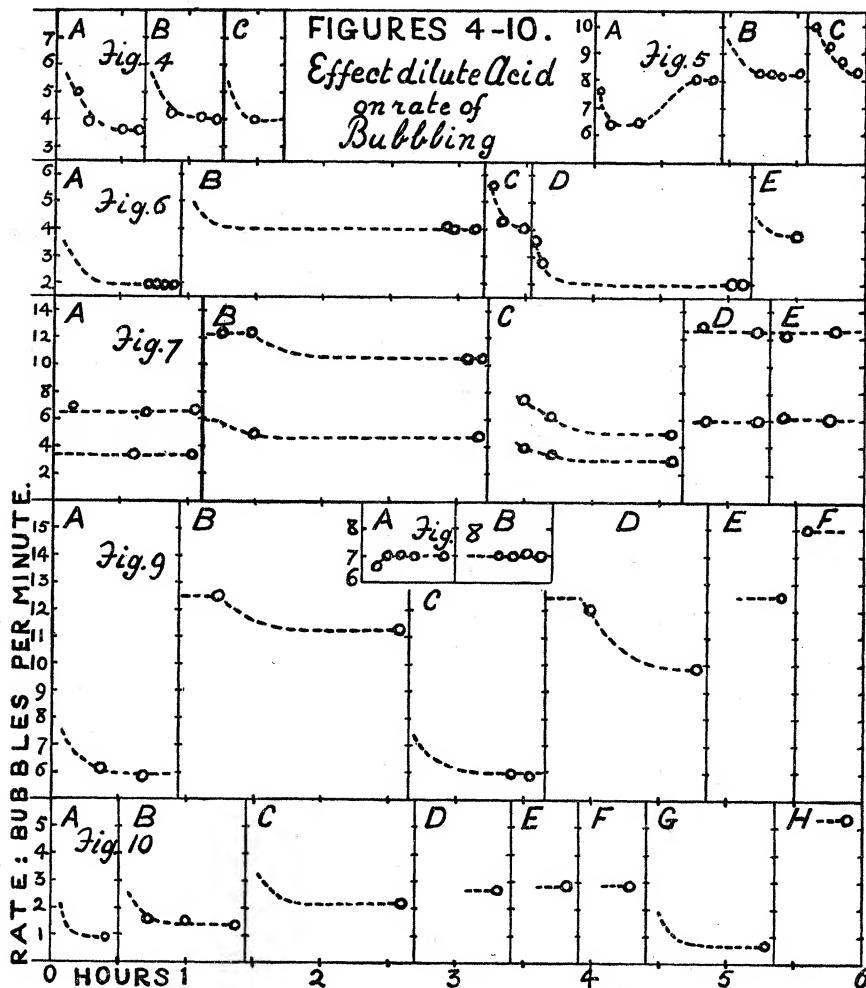


FIG. 4.—Elodea : Light intensity = 6* ; temperature 16° C. A, in 1 per cent. CO_2 solution† ; B, HCl added to 0.0005 N ; C, further HCl to 0.0015 N.

[In this and all other figures the broken line indicates the course of the bubble-rate that is to be assumed where there are no countings given.]

FIG. 5.—Elodea : Light intensity = 7.6 ; temperature 15° C. A, B and C, as in fig. 4.

FIG. 6.—Elodea from chalky stream : Light = 6 ; temperature 17.5°-18° C. A, 3.33 per cent. CO_2 solution ; B, HCl to 0.0005 N ; C, solution stirred but not

* Light intensity at 10 cm. from centre of burners is called arbitrarily = 10 ; other intensities were obtained after calculating by inverse square law the distance necessary to give the required value, the light being then set by means of a metre scale fixed alongside the apparatus. Values are thus only roughly approximate.

† All the strengths of CO_2 solution are expressed as volumes CO_2 at 0° C. and 760 mm. Hg contained in 100 volumes of solution.

In fig. 8 the CO_2 supply is well in excess of the light intensity, and no increased bubbling results on addition of HCl . In fig. 9 we have a more elaborate experiment, showing in its first part, sections A-B-C, a large effect of acid when weaker CO_2 is used, and in its second part, D-E, a small effect when stronger CO_2 is supplied; a third part, F, was carried out to demonstrate that it was really the limiting factor of light which kept the effect so small in E, for on simply bringing the light nearer, in F, the rate of bubbling rose at once.

In the next experiment of this series four separate shoots were set up to bubble in one large jar containing 500 c.c. of CO_2 solution; the results of acidification are tabulated below. Plant C came fresh from the chalk stream, A had grown in the water-tub all the winter, while B and B' were plants from the same habitat as C, but brought in some weeks earlier and transferred to the tub. The bubble rates of the individual plants in the one solution differed a good deal, partly determined by the sizes of the shoots and partly by the different sized nozzles of the four bubblers (C was a very short stout shoot, and its bubbler had a very large opening). To make them easily comparable, the actual rates have been multiplied by a factor, given at the head of the Table, to bring them all to the value of 10 for the original rate in CO_2 solution. On adding HCl to bring the acidity to 0.0008 N, the

changed; D, solution poured off and a second half of original 3.33 per cent. CO_2 solution put in; E, again HCl to 0.0005 N.

FIG. 7.—*Elodea* from chalky stream, two plants side by side in the same chamber—upper and lower records. Light = 12.3; temperature 18° C. A, in CO_2 solution; B, added HCl to 0.0010 N; C, second half of solution used in A; D, some strong stock CO_2 solution added to make the CO_2 very strong and superlimiting, in order to determine the light-limit value; E, HCl added to D to 0.0010 N; the light is now limiting and the effect of acid quite absent (see text).

FIG. 8.—*Elodea* from chalky stream in moderate light and strong CO_2 , so that light is limiting. Light = 6; temperature 18° C. A, 3.7 per cent. CO_2 solution; B, HCl to 0.0005 N.

FIG. 9.—*Elodea* from chalky stream: light intensity 12.3 in series A-E, in F 16. A, 0.65 per cent. CO_2 solution; B, HCl to 0.001 N; C, solution replaced by second half of the original 0.65 per cent. CO_2 ; D, 1.2 per cent. CO_2 solution; E, HCl to 0.001 N: here the light limit is reached before any large acid effect is manifest: the horizontal line at 12.5 b.p.m. marks the limiting light value; F, the light intensity is increased to 16 without altering the solution, and the bubble rate at once goes up to 15 b.p.m.

FIG. 10.—*Elodea* from chalky stream: Light = 12. A, in CO_2 solution; then in the five following stages additions of HCl were made to bring the acidity up to the following values; B, 0.00013 N; C, 0.00027 N; D, 0.00054 N; E, 0.0008 N; F, 0.00107 N. In G, the solution was changed for the second half of the solution used in A; the activity is not greatly depressed below the original value in A. In H, a greatly increased concentration of CO_2 was obtained by adding stock CO_2 solution, and the bubble rate goes up enormously.

chalk plant alone gives a positive reaction. It increased its rate fourfold, while the three shoots from the tub showed no increase. It will be seen that, after two hours in acid, the rate of bubbling in A, B, and B' has fallen to about half. It was not made clear to what extent possible escape of CO_2 from the solution, reduction of CO_2 concentration by assimilation, and injurious effect of the acid, were responsible for this big fall, as there was no opportunity of doing further experiments on this point. It is interesting to note that the extra source of CO_2 available for the chalk plant has come to an end, and that its rate also has fallen to half the original value. The termination of the temporary acid-chalk increase ought to be investigated further.

Experiment Table.

Elodea.		Grown in rain water.			From chalk stream.
4 shoots	Rate factor	A 7.8	B 18.3	B' 1.23	C 85.0
Time.		Relative rates of bubbling.			
p.m.					
12.8	10.0	—	—	10.0	
12.10	—	10.0	—	—	
12.13	—	—	—	—	
12.15	10.0	—	—	10.0	10.0
12.19	—	10.0	—	—	
12.20	4 c.c. N/10 HCl added to make acidity 0.0008 N.				
12.35	10.8	—	—	—	
12.36	—	—	—	10.2	40.0
12.38	—	11.5	—	—	
1.5	8.0	—	—	8.8	
1.9	—	10.0	—	—	39.2
2.10	—	—	—	—	
2.12	—	—	—	6.1	5.2
2.17	—	5.8	—	—	
2.23	5.5	—	—	—	
2.30	—	6.3	—	—	5.0
2.35	4.9	—	—	—	

The effect of dosing the plant, as Treboux did, with a series of increasing strengths of acid, is shown in fig. 10. In this case the first two increases of acid each produced a marked rise in bubble rate, but with subsequent increases the further rise diminishes until between E and F it is nil. That the value here reached is not merely a light limit is shown by the greatly increased rate in H when more CO_2 is given. In fig. 11 the rates of bubbling are plotted against concentration of acid, and it is seen that a linearly

increasing rate, at first, is followed by a uniform rate so that a curve like a CO_2 light limiting factor curve is produced. Knowing that the light limiting value is much higher, we cannot put this simple interpretation on the result. It must be remembered that it took four hours to carry through the series B to F, so that a time factor of some sort may be responsible for nullifying the direct effect of the stronger acids. It may be noted that in the Experiment table on p. 318 the acidification effect on C had fallen to nil in about two hours time, though this is not usually so. There was no opportunity of continuing the investigation and analysing out the factors that contribute to give the curve of fig. 11 its particular form.

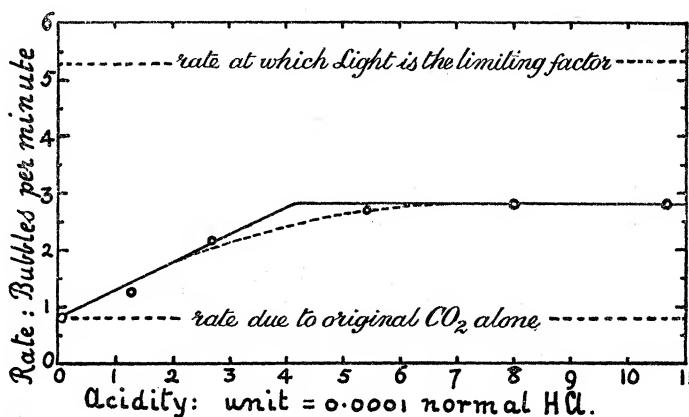


FIG. 11.—Effect of increasing acidity on bubble rate with Elodea. The data are taken from the experiment set out in fig. 10. It will be seen that, at the critical part of the curve, the data are not adequate to determine whether the points lie on a continuous curve or on two intersecting straight lines.

Part III.—COMPARISON OF ASSIMILATION RATES IN SOLUTIONS OF CARBONIC ACID AND OF SODIUM BICARBONATE.

Though several workers have studied the rate of bubbling of water plants in solutions of bicarbonates, no one has compared these rates of bubbling with the rates in known solutions of carbonic acid. With the aid of a bubbler and bubbling cup described in Part I, comparisons between different solutions can readily be made with the same plant, as a uniform bubble size is assured, whether the plant is in a solution of carbonic acid or of bicarbonate.

The results of Angelstein (1911), who observed bubble rates in a solution of bicarbonate which he gradually increased in concentration, are very obscure, especially because in all cases he chose to add CO_2 to the water before beginning the series of bicarbonate strengths.

For determining what strengths of CO_2 solutions are equivalent, in

assimilation capacity, with given strengths of bicarbonate, the ideal plan would be, after preliminary experiments, to be able to present results giving identical rates in a pair of selected solutions. Only a short time was, however, available for the experimental work of this section, and the equivalence of pairs of solutions had to be deduced by rule of three. For the soundness of this procedure it must first be demonstrated that in a series of CO_2 solutions of different strengths the rate of bubbling is directly proportional to the concentration within the range that it is proposed to use; the same proportionality should also be demonstrated for a series of bicarbonate concentrations. These two demonstrations are given in the next two sections, and the direct comparisons of CO_2 and bicarbonate follow in a third section.

(A) *Experiments with Carbonic Acid Solutions of Different Strengths.*

The results of the first experiment, in which the solution in the plant chamber was changed frequently for others of different strengths, is given in fig. 12. Each new solution shows the "initial CO_2 diffusion effect"/* leading to a steady rate. This steady rate in each solution would be maintained for

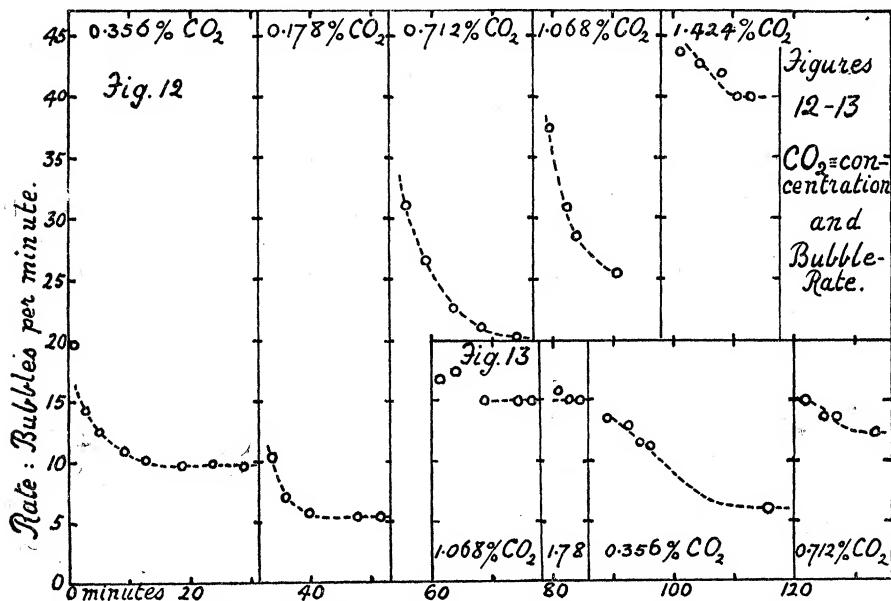


FIG. 12.—Bubble rates of *Callitricha* in a quickly changed series of CO_2 solutions, showing the initial CO_2 diffusion effect before the bubbling settles down to a steady rate. Strong light (= 10); temperature 17° C.

FIG. 13.—Similar series to that in fig. 12, but with a weak light of 3.5 units. Light now limiting in the first two solutions.

* See Part I, p. 310.

a long time, but readings were not continued in this particular experiment beyond the point necessary to determine what this steady rate was for each solution; for even thus the experiment occupied two hours. The final values are given in fig. 14 plotted against CO_2 concentration. They suffice to demonstrate that the bubbling rate is directly proportional to the concentration. This is not a new point, so that no elaborate experiments were made. Treboux demonstrated it, and it accords with the findings of Blackman and Smith (1911) using CO_2 analysis instead of bubble rates. It must be noted in fig. 12 that one of the values, the rate in the solution believed to be 1.068 per cent. CO_2 , is quite out of proportion by some error or other, but it was not thought worth while to repeat the series. In this experiment a strong light (intensity = 10) was used, and the light was in excess throughout. In the next series the light was reduced to 3.5 units, and a similar range of strengths of CO_2 was repeated.

These results are set out as a time sequence in fig. 13. In the last two strengths we have the same initial effects as with the strong light series, and here CO_2 is still limiting. In the first two strengths the CO_2 , however, is in excess, and the light limiting, and after some irregularities the final rate is

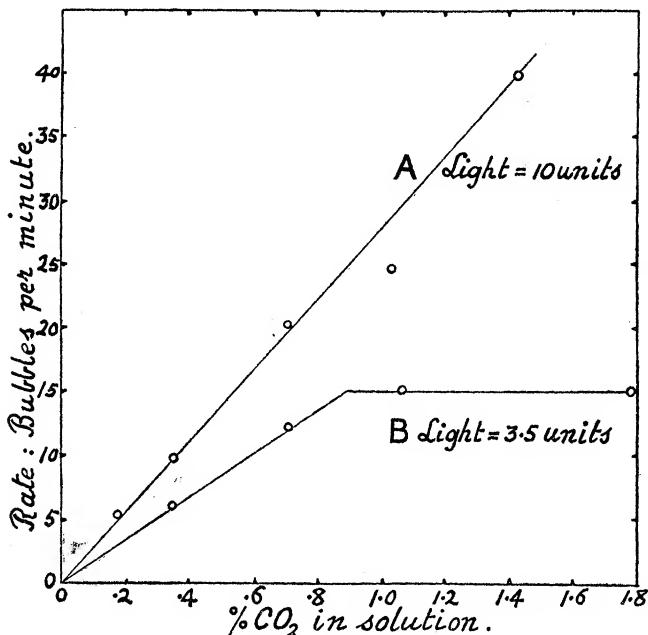


FIG. 14.—The final values adopted from figs. 12 and 13 are here plotted against CO_2 concentration. With weak light (3.5) the proportionality between concentration and bubble rate is cut off at about 0.9 per cent. CO_2 by the light factor, while with strong light (= 10) this proportionality is continued through the whole series tried.

attained without the falling curve. Here the CO_2 initial diffusion effect cannot reveal itself. The experimental record given is slight and brief and is only intended as an illustration. Throughout the work the relations of the factors light intensity and CO_2 concentration conformed to Blackman's exposition of the theory of limiting factors.

The final values for the four concentrations of fig. 13 are transferred to fig. 14, lower curve B, and it will be seen that they exhibit the form of the two-factor limiting curve. With weak light and strong CO_2 Treboux also records this type of curve.

This demonstration may suffice to establish that for our experiments, comparing CO_2 and bicarbonates, it is accurate to calculate bubble rates from one strength of CO_2 to another on the basis of direct proportionality; provided, of course, that care is taken to ensure that light is in excess throughout.

(B) *Experiments with Sodium Bicarbonate Solutions of different Strengths.*

Experimenting with series of bicarbonate solutions one does not encounter such simple regularity as with CO_2 solutions. After the initial CO_2 diffusion effect, which occurs here also, the rate is not maintained uniform for such long periods, but gradually declines. This is presumably to be attributed to the local alkalinity of the solution that must result from the removal of CO_2 . Angelstein records the injurious effect of added Na_2CO_3 in increasing the alkalinity, but this effect was not detached from other complications. Escape of CO_2 from the upper surface of the solution would increase the alkalinity of the liquid, so for the more precise experiments of the next section (C) a cork lid was employed to retard this effect. For the present section we have two experimental series, each of five increasing strengths of bicarbonate. At each strength of solution the plant and chamber were carefully rinsed, to remove adherent alkaline liquid. Such a series cannot be carried through in less than two hours, so to reduce the duration of the exposure, the bubble rates were only followed just to the end of the initial CO_2 diffusion effect in each solution. The final value adopted for each solution is not therefore as firmly established as if a long series of similar values had been counted. The time-sequences of these experiments are not detailed here, but the final adopted values for the two series are given in fig. 15. Both Series A and B were carried out with weak light (intensity = 3.5), but A with such weak bicarbonate solutions that the light limiting value was not reached. The points for Curve B, which is more extensive, are not very smooth, but the general relation of two limiting factors reveals itself here, as with carbonic acid. The data for A only presents the rising limb of the curve; presumably, had stronger solutions been used no higher bubble rate would have been

recorded. The points for A make a smooth sequence, but do not lie in a straight line: as the concentration increases, the bubble rate declines somewhat from direct proportionality. Whether this is a primary relation or is only an expression of the inflection of the curve, at the highest value, into its horizontal course has not yet been investigated.

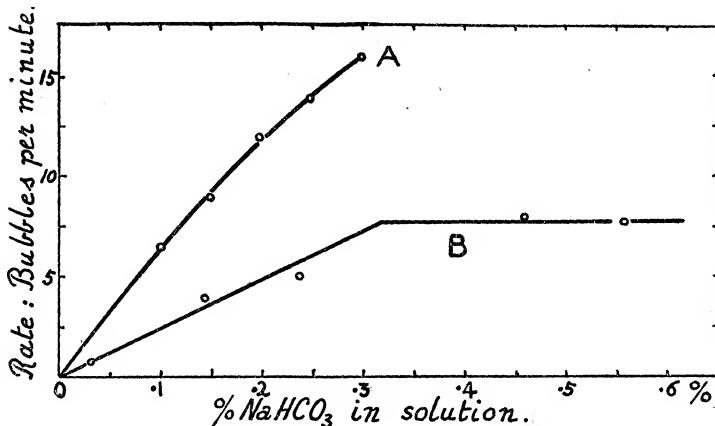


FIG. 15.—Rate of bubbling in sodium bicarbonate solutions of different concentrations. For both curves, light = 3.5. Curve A, one plant in five solutions of increasing strength; Curve B, another plant in five solutions covering a wider range. B shows a typical limiting factor curve, but A stops just where the horizontal limb would be expected.

Figs. 14 and 15 together enable us to compare the strengths of carbonic acid and sodium bicarbonate that *just* suffice to give the full effect that is potential in light of 3.5 units. These comparable values are given by the inflection points of the two B curves, both of which are for 3.5 light. Whereas 0.9 volumes of CO₂ per cent. is the critical value for CO₂, it is seen that 0.32 grm. NaHCO₃ per cent. is the corresponding value for bicarbonate. This provides us with our first equivalence ratio, $\frac{\text{ctn. bicarbonate}}{\text{ctn. CO}_2} = 0.35$.

This comparison is made very indirectly with two different plants at different times, and the location of the supposed inflection point is only got by interpolation. Direct comparisons set out in the next section show that 0.29 is a better value for this ratio.

The experiments described in this section, though not very searching, suffice to establish that for different concentrations of bicarbonate the bubble rates, if not in absolute direct proportion to the concentration, approximate closely to this relation, especially over a short range of concentrations.

(C) *Direct Comparisons of Sodium Bicarbonate and Carbonic Acid with the same Shoot.*

For the experiments in this section the rate of bubbling was first taken in CO_2 solutions, and then the plant was transferred to a bicarbonate solution. This sequence gives no risk of the readings in CO_2 containing an injurious after-effect of the bicarbonate solution. Fig. 16 shows the readings of the

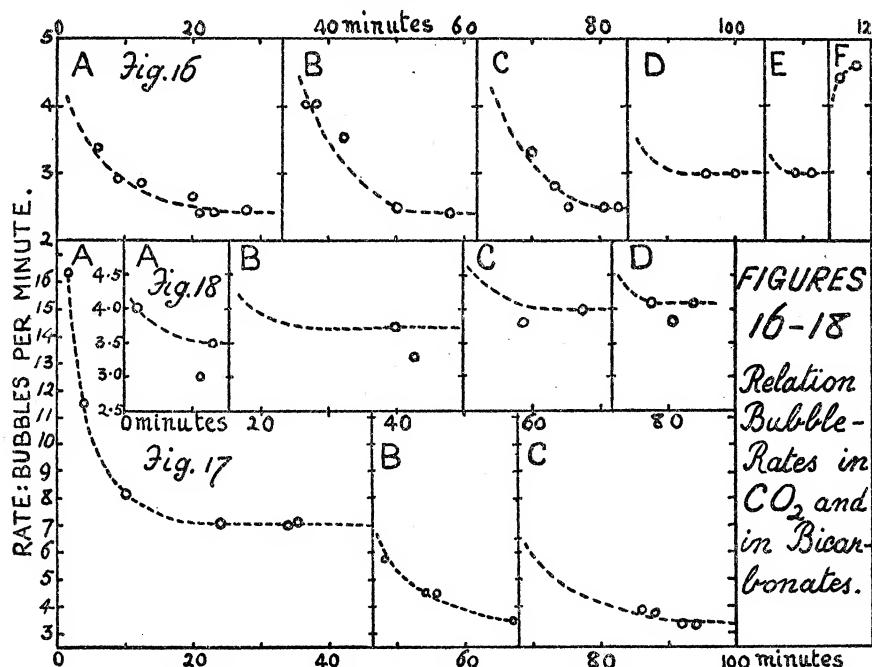


FIG. 16.—Bubble rates of Callitricha in six successive changes of solution. A, 0·6 per cent. CO_2 solution; B, fresh 0·6 per cent. CO_2 solution; C, 0·2 per cent. solution of NaHCO_3 ; D, fresh solution of the same concentration; E, ditto; F, 0·9 per cent. CO_2 solution. This last stronger solution was only employed to demonstrate that the values A-E are none of them limited by light.

FIG. 17.—Bubble rate of Callitricha. A, 0·6 per cent. CO_2 solution, the plant being observed for a long time in one solution instead of renewing the solution; B, 0·08 per cent. NaHCO_3 solution; C, fresh ditto.

FIG. 18.—Bubble rate of Callitricha. A and B, 0·6 per cent. CO_2 solutions; C and D, 0·166 per cent. NaHCO_3 solutions. This record shows the irregularity that may be got if the bubbler is not absolutely clean, so that some bubbles tend to stick on to the bubbler and increase to an abnormal size before breaking away. In such a case the resulting slow rates are neglected and the quicker values only adopted. This sticking recurs in each solution of this experiment.

first of these experiments. Each fresh solution shows its "initial CO_2 diffusion effect," leading to the final value representing the static efficiency of the solution. A and B give concordant values, 2·4 b.p.m. in 0·6 per cent. CO_2 ,

but C and D for 0·2 per cent. bicarbonate gave 2·5 and 3·0 b.p.m. respectively. A third sample, E, agreed with D in giving 3·0 b.p.m., and this rate, or the average of 2·8 b.p.m., may be adopted for 0·2 per cent. bicarbonate.

In fig. 17 are set out the data in evaluating the effect of a weaker bicarbonate solution. The rate in 0·6 per cent. CO_2 is 7·1 b.p.m., while in 0·08 per cent. NaHCO_3 it becomes 3·3 b.p.m. Fig. 18 presents the results with another strength of bicarbonate. Here duplicate solutions were used and the rates adopted were the quicker rates in B and D, namely, 3·8 b.p.m. in 0·6 per cent. CO_2 and 4·1 b.p.m. in 0·166 per cent. NaHCO_3 .

The bubble rates established for these three pairs of solutions are set out in the adjoining Table, as well as the adopted rates from two other experiments, which were rather irregular in some details.

The first use we can make of this set of ratios is to calculate from the CO_2 solutions what should be the CO_2 concentrations in the bicarbonate solutions, assuming that direct proportionality holds between rate and concentration in CO_2 . For the first case, the calculated CO_2 concentration of the bicarbonate

Experiment.	Assimilation in CO_2 solutions.		Assimilation in bicarbonate solutions.		Estimated CO_2 concentration percentage in bicarbonate solutions.	
	P.c. concentration.	Bubble rate.	P.c. concentration.	Bubble rate.	By assimilation rates.	Physico-chemically (extreme values).
a	0·60	7·1	0·08	3·3	0·28	0·24-0·28
b	0·30	0·5	0·10	0·5	0·30	0·31-0·36
c	0·385	4·0	0·10	3·5	0·34	0·31-0·36
d	0·60	3·8	0·166	4·1	0·65	0·53-0·60
e	0·60	2·4	0·20	3·0	0·75	0·64-0·72

solution equals $0·6 \times 3·3 / 7·1 = 0·28$ per cent. CO_2 . The similarly calculated values for all the other cases are set out in column 6. On this follows the question whether the CO_2 concentrations thus indicated biologically agree with the data derived from physico-chemical work on bicarbonates. Information on this point I owe to Dr. F. F. Blackman, who is dealing with this matter in another paper; he has supplied the numbers of the last column in the Table. These represent the extreme possible values of the CO_2 concentrations in the freshly made bicarbonate solutions used in these experiments. The ambiguity in these values is due to some of the constants involved in the calculation being insufficiently determined.

It will be seen that biological results align quite well with the physico-chemical, being close enough to establish the view that the rate of assimilation and bubbling in dilute bicarbonate solutions is due entirely to the concentration of carbonic acid that arises spontaneously by decomposition of

the dissolved salt. There is no support for Angelstein's view that such plants actively decompose bicarbonates.

SUMMARY AND CONCLUSIONS.

Part I.—A glass "bubbler" has been devised which, fitted on to the cut stem of submerged water plants, secures that the bubbles liberated in assimilation are of constant size, thereby doing away with one of the chief sources of trouble with this procedure. When, in addition, this bubbler delivers bubbles into an isolated "bubbling cup" of distilled water, the bubbles are removed from the direct influence of the solutions that are being experimented with (see fig. 1, p. 307). In this way alterations of surface-tension by added solutes are eliminated, and any osmotic effects on the cells of the cut surface do not change the size of the bubbles.

The disturbances of bubbling rate due to the "initial oxygen diffusion effect" and the "initial CO₂ diffusion effect" are explained and illustrated. The former (see fig. 2, p. 310), due to inadequate oxygen content of the solution, is of widespread occurrence with other workers, but is eliminated in this work by using water heavily charged with oxygen. The CO₂ effect is the cause of the phenomenon of initial high bubbling rates which rapidly decline (see fig. 3, p. 310). It marks the initial stage before a steady static diffusion gradient of CO₂ is set up. This effect cannot be eliminated when CO₂ is a limiting factor, but does not appear if light intensity is limiting. As these two initial diffusion phenomena are independent and of opposite effect, they may in some cases mask one another mutually, and give a record which does not reveal the initial disturbances.

Part II. Action of Acids upon Assimilation by Submerged Water Plants.—It is shown that the augmentation of bubble rate which Treboux has recorded on adding acid is due to the acid setting free CO₂, locally, from calcium carbonate incorporated in the surface of plants growing in chalky waters. No such acid effect is shown by the same plants when grown in soft water.

That the action of the acid is merely to set free an additional supply of CO₂ is proved by the fact that, with plants grown in hard waters, acid only produces an increase of bubble rate when CO₂ supply is the limiting factor to the rate of bubbling at the time the acid is added. If the plant is placed in relatively strong CO₂ and weak light, so that light is limiting, the addition of acid has no effect upon the rate of bubbling. This evidence seems to dispose of the suggestion that increased bubbling rate with acid is due to CO₂ liberated from the adsorbed state and escaping as a CO₂ addition to the volume of the bubbles. This type of effect should not disappear when light is limiting.

Part III. Comparison of Assimilation Rates in Solutions of Sodium Bicarbonates and of Carbonic Acid.—By the aid of the “bubblers” described in Part I these rates are carefully compared for solutions of known strength. It is shown that a solution of a bicarbonate gives just about that rate of bubbling which corresponds to the CO₂ concentration expected to arise in it by spontaneous decomposition. Solutions of bicarbonates, when very dilute, give initial CO₂ diffusion effects, conform to Blackman's laws of interaction of limiting factors, and generally behave as solutions of carbonic acid. Angelstein's view that plants have the power of splitting bicarbonates actively is erroneous.

In conclusion I wish to express my thanks to Dr. Blackman, both for advice during the course of the experiments and for help in preparing this paper when stress of official duties, preventing me from giving the necessary attention to it, had resulted in an indefinite postponement of publication.

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